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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/802,875	LIEW, CHOONG-CHIN	
	Examiner	Art Unit	
	Juliet C. Switzer	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 November 2007 and 08 January 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *45,46,48,49 and 52-75* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *45,46 and 54-75* is/are rejected.

7)☒ Claim(s) *48,49,52 and 53* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. This action is written in response to applicant's correspondence received 11/16/07 and 1/8/08. Claims 45-46, 48-49, and 52-53 have been amended, claims 47, 50, and 51 have been canceled, and claims 54-75 have been added. Claims 45-46, 48-49, and 52-75 are pending are examined herein. Applicant's amendments and arguments have been thoroughly reviewed, but are not persuasive to place the claims in condition for allowance for the reasons that follow. Any rejections not reiterated in this action have been withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action. **This action is FINAL.**

2. The declaration filed 11/16/07 and again 1/8/08 has been considered and is discussed in the response to remarks section.

Claim Objections

3. Claims 48, 49, 52, and 53 objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim cannot depend from another multiply dependent claim. Each of these multiply dependent claims depends from multiply dependent claim 56. See MPEP § 608.01(n). Accordingly, the claims not been further treated on the merits.

4. Claims 60, 61, 65, 66, 67, 68, 69, 70, 71, 72, 73 and 74 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the

application was filed, had possession of the claimed invention. This is a rejection for new matter.

5. Claim 60 appears to have new matter. The specification does not provide basis for a claim which broadly states that any time a test subject's RNA expression of ABCA1 is "lower" than the expression of healthy control subjects that the subject is a candidate for having coronary artery disease. Regarding the expression of ABCA1, the specification provides only very limited data, while this claim specifically recites "lower" expression values. The specification, in table 3L provides a list of genes that were differentially expressed between a group of patients who have CAD and a group of healthy controls. The specification on page 82 teaches that genes were identified as differentially expressed with a p value of <0.05. ABCA1 is among the listed genes, and Table 3L teaches that the p-value for the comparison is 0.046748641. The specification also teaches that ABCA1 is differentially expressed in patients having schizophrenia and patients having Chagas' disease relative to normal controls (Tables 3Y and 3Z). Table 3L lists genes that were differentially expressed, but does not provide any further information regarding the level of expression. For example, the tables do not teach if the expression was higher or lower in coronary artery disease patients versus controls. Thus, the broad statement in the claim regarding classifying the subject as a candidate for CAD if the RNA level "is lower" appears to be new matter.

Furthermore, claims 61, 65, and 71 also have new matter because they not only recites that the expression is lower, which is problematic for the previously stated reasons, but even more specifically states that it is 1.5 times lower, for which no basis has been identified in the specification.

Likewise, claims 62, 66, and 72 have new matter because they recite that the level is 1.54 times lower than that of the control subjects and no basis has been identified in the specification for this value.

In claims 64, 66, and 72 the limitation "with a p value equal to 0.0001" appears to be new matter. Applicant did not identify basis for this limitation in the response and the examiner was unable to identify basis for the limitation.

6. In claims 67, 68, 69, 70, and 73, the limitation that the blood samples "comprises leukocytes which a have not been fractionated into cell types" is new matter. Such a recitation includes, for example, testing a blood sample where the red blood cells and the white blood cells have been separated, and also includes, the testing of whole blood RNA. There is clearly basis for the latter, but not the former. Applicant asserts in the remarks that this claim limitation finds clear support in the specification, including figure 5C which shows standardized fractions of leukocytes. However, these are not leukocytes that have not been fractionated into cell types, as they have clearly been fractionated into cell types. While RNA levels have been determined in each of the fractions, this is not basis for the negative limitation "have not been fractionated into cell types." There is no discussion or example in the specification of the testing of RNA in blood samples which comprise leukocytes which have not been fractionated into cell types. Applicant has attempted to present a claim which excludes a particular process step from a method (that is, fractionating the leukocytes) and then provides basis for the exclusion of the step in a method where the opposite occurred. This is not sufficient basis for the claim limitation because there is nothing in the specification that suggests applicant contemplated the exclusion of a step of

fractionating leukocytes into cell types. Therefore, the claims are rejected for having new matter.

All claims which depend from the specifically discussed claims are rejected for having new matter because of their dependency from the specifically enumerated claims.

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

8. Claims 54, 56, 57, 58, 59, 67, 68, and 69 are rejected under 35 U.S.C. 102(a) and 102(b)as being anticipated by William Chittenden, dissertation submitted to the faculty of Virginia Polytechnic Institute and State University, August 2002.

9. These claims are not fully supported under 112 1st paragraph in the instant application nor any of the previously filed applications for at least the reasons discussed in this office action. This reference is applied under 102(a) and 102(b). If applicant establishes support for the claimed invention to a prior application such that the 102(a) and/or 102(b) does not apply the rejection will be withdrawn.

Chittenden teaches quantification and analysis of gene expression in mRNA isolated from whole blood, by isolating cells, precipitating RNA, producing cRNA and hybridization with the probe array HG-U133A, quantification of hybridization and calculation of differential expression. It is an inherent property of this array that it contains probes to ABCA1, and thus,

the method taught by Chittenden is a method which uses an oligonucleotides of predetermined sequence which are specific for ABCA1. This chip also inherently has thereupon housekeeping genes that are used for quantifying expression. Further Chittenden tests individuals with disease and healthy controls (p. 58-59, 62-66). Chittenden does not specifically discuss ABCA1 expression, but it would have inherently been detected in the blood of healthy controls by the hybridization and array reading methods.

10. Claims 54-59, 67, 68, 69, are rejected under 35 U.S.C. 102(b) as being anticipated by Lawn et al. (WO 00/78971, as cited in IDS).

Lawn et al. teach a method for detecting expression of an ABCA1 gene (referred to therein as ABC1) in a human test subject comprising detecting RNA encoded by said gene in a blood sample of said test subject (p. 68, lines 10-30). In particular, Lawn et al. teach that the mRNA expression can be assayed using methods in Examples 2 and 9. These examples use microarray analysis and quantitative RT-PCR, respectively, both methods which use an oligonucleotide of predetermined sequence which is specific only for RNA encoded by ABCA1 gene. The RT-PCR method comprises producing an amplification product with primers specific only for RNA encoded by ABCA1 gene. Both of the methods in examples 2 and 9 include quantifying a level of RNA encoded by said gene in said sample. Lawn et al. teach comparing a level of RNA with a pre-determined standard level of mRNA expression obtained from subjects that do not have CAD (i.e. are healthy subjects). Regarding claims 67, 68, and 69, the blood samples taught by Lawn et al. are considered "whole blood samples" because, all blood samples begin as whole blood samples. The open claim language allows for additional steps to be included in the method, in this case the enrichment of the monocyte population.

This reference has not been applied to claims 60-64 because, although Lawn et al. teach that determination of a decreased level of ABC1 mRNA in the test sample relative to the standard level can be used to indicate a susceptibility to CAD in the subject, Lawn et al. do not appear to provide any data to support this assertion for humans and blood samples, and so, for the steps of classifying, Lawn et al. do not appear to be enabled.

Claim Rejections - 35 USC § 103

11. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claims 71, 72, 73, and 74 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lawn et al.

Lawn et al. teach a method for detecting expression of an ABCA1 gene (referred to therein as ABC1) in a human test subject comprising detecting RNA encoded by said gene in a blood sample of said test subject (p. 68, lines 10-30). In particular, Lawn et al. teach that the mRNA expression can be assayed using methods in Examples 2 and 9. These examples use microarray analysis and quantitative RT-PCR, respectively, both methods which use an oligonucleotide of predetermined sequence which is specific only for RNA encoded by ABCA1 gene. The RT-PCR method comprises producing an amplification product with primers specific only for RNA encoded by ABCA1 gene. Both of the methods in examples 2 and 9 include quantifying a level of RNA encoded by said gene in said sample. Lawn et al. teach comparing a level of RNA with a pre-determined standard level of mRNA expression obtained from subjects

that do not have CAD (i.e. are healthy subjects). Regarding claim, the blood samples taught by Lawn et al. are considered "whole blood samples" because, all blood samples begin as whole blood samples. The open claim language allows for additional steps to be included in the method, in this case the enrichment of the monocyte population.

Lawn et al. do not expressly teach detecting RNA encoded by said gene in blood samples of human patients diagnosed with having CAD and comparing that to quantified level of control RNA encoded by said gene in blood samples of healthy control subjects. However, given the express suggestion by Lawn et al. to use such an assay as a test for CAD, it would have been prima facie obvious to one of ordinary skill in the art to have tested populations of patients with CAD and healthy patients to determine the level of ABCA1 expression in blood samples in order to establish the validity of the assay taught by Lawn et al. One would have clearly been motivated by the teachings of Lawn et al. to develop a blood based test for indicating susceptibility for CAD, a common human disease. One would have been motivated to set experimental ranges for the level of differences to be observed, and to use standard statistical methods including p-value cut offs of 0.05 or 0.0001 because these indicate different levels of error and evaluating experimental data. Further, the level of difference observed would be an inherent property of the practice of the method. Thus, in view of Lawn et al. the claimed invention is prima facie obvious.

Claim Rejections - 35 USC § 112

13. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

14. Claims 45-46, 54-70, and 75 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Nature of the invention

Claim 45 is drawn to a method detecting coronary artery disease in a human test subject, and feature a step of quantifying a level of RNA encoded by an ABCA1 gene in a blood sample from a single test subject and comparing the level with a quantified level of RNA encoded by said gene in blood samples from control subjects who are classified as healthy control subjects, and comparing the level of RNA in the sample with control subjects who are classified as having coronary artery disease (CAD). Claim 45 sets forth that a determination of a statistically significant similarity between the test level and the level of control subjects having said coronary artery disease and a statistically significant difference between the test level and the level of healthy control subjects "is indicative of said coronary artery disease."

The nature of the invention requires the knowledge of a reliable association between comparing ABCA1 expression and the indication that coronary artery disease is present in a human. Further, the practice of the invention requires an understanding of how the presence of coronary artery disease effects the level of CRTAM expression in human blood.

Claim 54 is drawn to a method for detecting expression of a gene encoding a ABCA1 in a human "test subject." Claims which depend from claim 54 set forth that the detected expression is quantified and compared to quantified level of control RNA encoded by said gene in blood

samples of control subjects. Listed control subjects include healthy subjects and subjects having CAD. Further dependent claims set forth steps of classifying or identifying the test subject as being a candidate for having CAD depending on the outcome of the comparing steps. Thus, it is clear that the intended use of claim 54 and those that depend from claim 54 is for classifying or identifying the test subject as being a candidate for having CAD. Claims 60, 61, and 62 recite that if the level of expression is "lower" than healthy control subjects, then the individual is a candidate for CAD, with claims 61 and 62 reciting at least 1.5 times lower and 1.54 times lower.

Independent claim 65 sets forth a method for screening a human test subject for having CAD and includes similar detection, quantification, and comparing steps, reciting that a test subject is a candidate for having CAD if said level of RNA encoded by said gene in said blood sample of the test subject is "at least 1.5 times lower" than that of said healthy control subjects with a p value <0.05. Claim 66 is similar, but recites that the subject is a candidate for having CAD if the level of RNA encoded by said gene is 1.54 times higher than that of said control subjects classified as healthy subjects with a p value equal to 0.0001.

The nature of the invention requires the knowledge of a reliable relationship between ABCA1 expression in blood and the presence of CAD.

In claim 75, the invention is drawn to a method a method for classifying ABCA1 gene expression in a human, and sets forth steps of quantifying a level of RNA encoded by an ABCA1 gene, comparing that level to a level of RNA found in blood samples from control subjects having CAD and also comparing it to control subjects who are healthy. The independent claim states that based on particular determinations, the classification of ABCA1 gene expression results either with that of said subjects having CAD or with that of subjects who are healthy.

The nature of the invention requires the knowledge of a reliable association between ABCA1 expression and the ability to classify a particular individual's expression with the expression of subjects having CAD or not having CAD, and further, the use of this method requires that there is an underlying assumption that this classification is meaningful. Reading the claims in light of the specification it is clear that applicant intends to use such a classification method in order to provide a tool that is used as part of a diagnostic process, and such a use requires the knowledge of a reliable association underlying the classification. Further, the practice of the invention requires an understanding of how the presence of CAD affects the level of ABCA1 expression in human blood in patients having CAD versus patients that do not have CAD but may have some other disorders.

Many of the claims additionally require a step of comparing the level of RNA detected in a test subject to "a quantified level of control RNA encoded by said gene in blood samples of control subjects." To practice these claims, it is essential to know the quantified level of control RNA encoded by said gene in blood samples of control subjects.

Scope of the claims

Many aspects of the claims remain quite broad.

In some claims the health status of the control individuals is entirely undefined, and encompass subjects with CAD, healthy patients, patients with some other disease, such as such as Chagas disease or schizophrenia.

Many of the claims are very broad in scope because they encompass that ANY level and direction of difference in gene expression between the healthy controls is indicative of said

CAD, if that difference is "statistically significant." That is, the claims do not set forth that one level should be higher or lower than the other, and further do not set forth how much of a "difference" between two individuals would be necessary to draw the conclusions set forth in the claims. Many claims recite that a difference is identified but do not require that the difference is statistically significant at any particular level, and so, any level of difference observed can result in classifying the test subject as a candidate for disease. These claims do not recite a level of statistical significance that is required to be reached, and so, the claims remain quite broad since no particular level is required, and the claims even encompass using different levels of statistical significance for different comparisons. The phrase "statistically significant" describes a mathematical measure of difference between groups, not a particular level of difference which is acceptable. There is no universally accepted level of "statistically significant."

Claim 66 is representative of the narrowest claims set forth in the instant claim set, but the relationship set forth in this claim is not supported by the specification, as noted in the new matter rejection in this office action. This claim specifically defines the control population as healthy subjects and sets forth a very particular ratio of gene expression in the test subject relative the healthy control subjects.

Teachings in the Specification/Examples

Regarding coronary artery disease, the specification provides examples 9 and 21 wherein gene expression profiles of blood samples from individuals having coronary artery disease were compared with normal individuals, that is healthy patients. Example 9 teaches that 108 different genes were differentially expressed, but ABCA1 was not one of these. Example 21 teaches that 967 genes were identified as being differentially expressed, and regarding the instant claims,

table 3L provides a list of these genes (Example 21). ABCA1 is among the genes. The specification also teaches that ABCA1 is differentially expressed in patients having schizophrenia and patients having Chagas' disease relative to normal controls (Tables 3Y and 3Z).

Table 3L lists genes that were differentially expressed, but does not provide any further information regarding the level of expression. For example, the tables do not teach if the expression was higher or lower in coronary artery disease patients versus controls. Some claims being currently examined set forth that the expression is "lower" with some reciting particular levels, but these limitations are not supported by data in the specification.

The specification does not provide any guidance as to the level of "difference" that is sufficient (1 fold, 2 fold, etc) to result in a conclusion that coronary artery disease is detected, nor does the specification provide any guidance as to the direction of the difference (higher or lower expression) that is expected to be observed for any single pairing of samples.

The specification fails to provide information about an essential aspect of the invention, namely, the nature of the difference in expression that was observed between coronary artery disease patients and healthy patients. Furthermore, though the specification teaches that this gene is differentially expressed in coronary artery disease patients versus healthy patients, the specification teaches this is true for hundreds of genes, and the specification teaches this is true for this gene and two other diseases. There is no guidance or analysis of data in the specification to suggest that this gene in particular is sufficient to conclude that coronary artery disease is present in a sample, as is instantly claimed. This information is essential to understanding and

practicing the claimed invention because it is critical to knowing how to interpret a particular comparison result.

State of the Prior Art and Level of Unpredictability

The expression of genes in example 21 was tested by hybridization of samples to a microarray that contains genetic information for tens of thousands of genes. This technology area is highly unpredictable, and as a result significant guidance is required to practice inventions using this type of data. Lee (Clinical Chemistry, 47:8, 1350-1352 (2001)) teaches that despite the technical accuracy of individual observations on an array, these data "are much more prone to numerous false-positive findings fundamentally because of (a) an extremely large number of observations and (b) a very wide dynamic range of gene expression values obtained from gene chip experiments." In view of these unpredictable aspects of applying such data, Lee teaches that replication is necessary to begin to screen out false positive results. There is no replication in the instant specification.

Albrecht et al. did not observe a difference in expression of ABCA1 gene in blood cells from patients having carotid artherselerotic plaques (that is coronary artery disease) versus healthy patients. Thus, at least in the population and assay Albrecht et al. carried out, it appears that the assertion in the instant claims, that comparison of blood sample levels of ABCA1 mRNA) was not replicated. This is further evidence of the highly unpredictable nature of this technology area.

Furthermore, there is no analysis of all possible diseases or phenotypes to determine if the gene expression difference observed in the instant application is specific to coronary artery disease such that any difference between a test patient and blood samples from control subjects is

sufficient to conclude coronary artery disease is present. The instant specification in fact teaches that ABCA1 is also differential expressed in the blood of patients having schizophrenia or Chagas disease relative to healthy controls. So first, even if one carried out the claimed analysis on a test subject, and if one observed a level of expression, it is highly unpredictable how would one begin to know if that level of expression indicated coronary artery disease, Chagas disease, schizophrenia, all three, one but not the others, something in between or even some other condition or disorder for which the expression profile has not yet been determined. It is unknown and unpredictable whether it would be expressed in the blood of patients having other cardiovascular diseases or any other diseases which were not tested in the instant specification or diseases which were tested in the instant specification but in a different population of test subjects, and whether this expression would be different from levels of expression in healthy controls. A method for detection which relies on a comparison between expression in the blood of a test subject and control subjects requires the knowledge of this information in order to reliably "detect" coronary artery disease, as set forth in the claims. The instant specification has not established that all difference, no matter the magnitude nor the direction, relative to any control subjects or even relative to a healthy control subject is indicative of coronary artery disease. It is not known under what circumstances the result observed in the instantly examined control and test populations would be repeatable, as the results have not been validated. But even if one were to obtain the same result, it would be unknown because applicant did not disclose the magnitude of difference in expression between coronary artery disease patients or controls, nor did applicant disclose the direction of variation. All of these inquiries are

particularly important in this case since the specification is silent as to which differential expression observations would be sufficient to detect the presence of coronary artery disease.

Further, the claims of the instant application set forth the comparison of the gene expression in a single individual versus as few as two other individuals, and they set forth that a comparing gene expression between the two is "indicative of" coronary artery disease. Neither the specification nor the claims set forth a threshold of difference between an individual's expression and the control expression of ABCA1 in the blood that would be sufficient to conclude that the difference in gene expression between a test individual and any type control group is "indicative of" recited coronary artery disease. Because the claims encompass any level of altered gene expression, it is relevant to point out that the art of Cheung et al (2003) teaches that there is natural variation in gene expression among different individuals. The reference teaches an assessment of natural variation of gene expression in lymphoblastoid cells in humans, and analyzes the variation of expression data among individuals and within individuals (replicates) (p.422, last paragraph; Fig 1). The data indicates that, for example, expression of ACTG2 in 35 individuals varied by a factor of 17; and that in expression of the 40 genes with the highest variance ratios, the highest and lowest values differed by a factor of 2.4 or greater (Fig 3). It is thus unpredictable as to whether or not any level of altered gene expression is indicative of a coronary artery disease or the absence of coronary artery disease.

The unpredictability of correlating gene expression level to any phenotypic quality is taught in the post-filing art of Wu (2001). Wu teaches that gene expression data, such as microarray data, must be interpreted in the context of other biological knowledge, involving various types of 'post genomics' informatics, including gene networks, gene pathways, and gene

ontologies (p.53, left col.). The reference indicates that many factors may be influential to the outcome of data analysis, and teaches that expression data can be interpreted in many ways. The conclusions that can be drawn from a given set of data depend heavily on the particular choice of data analysis. Much of the data analysis depends on such low-level considerations as normalization and such basic assumptions as normality (p.63 - Discussion). The art of Newton et al (2001) further teaches the difficulty in applying gene expression results. Newton et al. teaches that a basic statistical problem is determining when the measured differential expression is likely to reflect a real biological shift in gene expression, and replication of data is critical to validation (p.38, third full paragraph). There is no replication of data in the instant specification.

Quantity of Experimentation

The instant specification does not provide enabling support for the practice of a single embodiment within the claimed invention. In particular, the specification does not provide adequate guidance to appraise one of ordinary skill in the art as to what levels of ABCA1 gene expression must be observed to successfully conclude that coronary artery disease is present. Further, although the specification teaches there are differences in ABCA1 levels in a coronary artery disease population versus a control patient population, the specification is silent as to the nature of the "difference" in magnitude or direction. Thus, given the lack of teaching in the specification and the highly unpredictable nature of the technology, an extensive amount of work would be required to practice the claimed invention.

In order to practice the claimed invention, one would have to undertake an extensive amount of experimentation in a highly unpredictable technology area. One would begin by trying to reproduce the results observed in the instant specification to determine if there is a

relative upregulation or downregulation of ABCA1 in coronary artery disease patients versus healthy control patients, as the specification does not even provide this minimal guidance. Without this knowledge one would not even begin to know how to interpret any results obtained in practicing the claimed methods. For example, consider the comparison of a test result and a control population of healthy individuals. How different from the average level of expression of healthy individuals would the test result have to be to indicate coronary artery disease? Would any difference, up or down regulation be indicative of coronary artery disease? Or could one indicate coronary artery disease and one a different undisclosed disease or schizophrenia or Chagas disease? Is ABCA1 expressed in the blood of individuals with a disease other than schizophrenia, Chagas disease and coronary artery disease? Is this expression also diagnostic of other diseases of the cardiovascular system or other disorders entirely unrelated to coronary artery disease? In order to reliably use a method for detecting coronary artery disease, one would first have to answer at least these questions. One would also, however, have to carry out this testing for validation, for it is possible that the result observed in the instant specification is intrinsic to the cohort of patients evaluated in applicant's study. Further, one would have to undertake experimentation to determine difference thresholds required to determine that a patient has or does not have a disease.

As discussed, this art area is highly unpredictable.

Conclusion

The claims include methods which encompass the detection in blood of the expression of ABCA1 in a test subject and comparing this expression to control subjects, wherein the comparison itself "is indicative of coronary artery disease." The identification of gene

differential expression/disease indication relationships is a highly unpredictable endeavor, requiring extensive experimentation. The specification provides minimal guidance. In light of the factors discussed, therefore, it is concluded that it would require undue experimentation to practice the claimed invention.

Although some of claims are drawn to a method of "detecting expression" or "classifying expression," and not to diagnosis or identifying increased likelihood of disease or the like, it is critical to understand how the classification can be used in order use the claimed invention. In this case, the specification does not provide sufficient guidance as to how to use the detecting or classification methods other than in methods that are directed towards diagnostic purposes. What is the meaning of classifying expression "with that of subjects having" CAD or with subjects who are healthy? While one could do the method steps as written, thus satisfying the "how to make" aspect of 112 1st paragraph, the specification does not provide sufficient disclosure to satisfy the how to use aspect of the requirement.

The data in the specification is not replicated. As discussed in the rejection, it is established that the technology on which the instant claims is based is a highly unpredictable technology, and in the face of such a high level of unpredictability, replication is necessary before results can be considered sufficient to support claims directed at classifying the gene expression of an individual test subject. Therefore, even this claim, after having considered all of the factors set forth in this rejection, lacks proper enablement.

Response to Remarks

Applicant traverses the rejection for lack of enablement. Applicant traverses the rejection insofar is it applies to the pending claims, beginning on page 13 of the response.

Applicant states that the instant claims recite three clearly defined sets of controls. Not all claims are so limited (see for example claim 57).

Applicants point out that claims which recite that the test subject is a candidate for CAD if the level of RNA encoded by ABCA1 is "lower" than that of healthy subjects, requiring in some, but not all cases, a level of statistical significance of $p<0.05$. However, while this is significantly narrower than the previously pending claims, the limitations are new matter and are not enabled by the specification since the specification is silent as to the difference or magnitude of direction which was observed between the population of healthy subjects and subjects with CAD.

Applicant states that ABCA1 is indeed sufficient to provide an indication of CAD on the grounds that the specification discloses that RNA encoded by the ABCA1 gene in a blood sample for a CAD patients is differently expressed relative to healthy subjects. First, applicant did not show that a statistically different level was observed between a single individual's level of expression and healthy patients, as stated in the response. Further, the instant specification fails to provide a critical piece of information with regard to understanding the relationship between ABCA1 expression and CAD. The specification invites one of skill in the art to undertake experimentation to (a) determine the relationship between CAD and ABCA1 expression and then to (b) validate that relationship. There is a fundamental absence of information given in the specification. The claims all set forth comparing the test level to "a quantified level of RNA encoded by said gene in blood samples from control subjects..." but the specification does not provide this quantified level, or any quantified level. So, it is left to one of skill in the art to establish what is critical for the practice of the invention. While the

specification may rely on the state of the prior art to help enable the invention, the specification may not rely on the state of prior art to supplement what is critical to the practice of the invention- in this case the quantified levels of control RNA encoded by the gene in the control subjects, no matter which type of control subjects.

Applicant points to the declaration which discloses post-filing validation using quantitative RT-PCR and an independent cohort of 14 healthy control subjects and 19 subjects having CAD. Applicant points out that the declaration clearly shows that RNA encoded by the gene ABCA1 is present at a statistically lower level in blood of subjects having CAD relative to healthy control subjects.

First, it is presumed that in paragraph 3 of the declaration applicant intended to refer to patients having coronary artery disease and not bladder cancer.

The declaration demonstrates that ABCA1 has significantly lower expression in CAD patients, but this does not make up for the deficiency in the specification. The experimental results disclosed in the declaration add to the teachings in the specification since they teach that ABCA1 RNA have been experimentally shown to be significantly lower in CAD patients relative to healthy controls. The claims all rely on comparison to ABCA1 quantified levels that are not given in the specification.

It is not known, and unknowable from the specification if the level of expression in other diseases (such as other cardiovascular diseases) is the same as that for coronary artery disease patients. Likewise, as pointed out in the rejection this gene is differentially expressed in blood of patients with schizophrenia and Chagas disease, and so it is not known if this level is the same or different as those patients with coronary artery disease. Some of the claims recite that they are

methods for "**detecting**" coronary artery disease, and so in order to detect the disease one must be able to put the result into a larger context.

Applicant states that because of the guidance in the specification which shows a statistically significant correlation between the levels of ABCA1 RNA in blood of diseased versus healthy controls, applicant contends that one of skill can reasonably predict that a patient may be a candidate for coronary artery disease based on the teachings of the specification. This is not persuasive for reasons previously discussed in the rejection. Following the guidance in the specification one would merely be invited to replicate what applicant has done with a hope to obtain the same result, but no way of knowing if one had done so.

Applicant disagrees that Albrecht et al. does not show the unpredictability of the claimed invention because thy use a minimum fold change of 2 fold which would not have detected the difference of 1.5 lower levels disclosed in the declaration. This argument relies on the findings in the declaration which are not provided in the specification. Further, this argument exemplifies the unpredictability of the technology. There is no guidance in the specification to suggest that for ABCA1 and CAD the difference is a 1.5 fold difference, and the level of difference expected between the two populations is impossible to predict absent specific guidance. Thus, given the guidance in the specification it clearly appears that Albrecht et al. failed to duplicate the findings of the example in the specification. Also, Applicant's argument is not commensurate in scope with most of the claims which encompass determining any level of difference, provided it is statistically significant.

On page 16 of the response, applicant asserts that the declaration replicates the findings of the specification. However, it is not knowable from the teachings in the specification if the

data actually replicates the findings in the specification since the specification does not give complete data. It is unknown if the relationship observed in the experiment provided in the declaration is the same as the relationship observed using the microarray analysis. One cannot make this comparison because the data given in the specification are incomplete. Based on the disclosed fact pattern in the instant specification, one could not extrapolate that ABCA1 expression is sufficient to "detect" coronary artery disease, as set forth in the claims. On cannot readily extrapolate whether or not the level of ABCA1 is the same or different in coronary artery disease and other diseases such as Chagas disease or schizophrenia. If the levels are the same, it would not be sufficient to show that ABCA1 expression is the same as a patient with coronary artery disease in order to detect coronary artery disease. One cannot readily extrapolate that the observation made in the specification is the same universally and not cohort specific since no specific guidance is given in the specification. One cannot readily extrapolate one could successfully differentiate different types or stages of coronary artery disease based on the disclosed data.

Applicant disagrees with Wu that expression data needs to be interpreted in view of other biological knowledge. Wu was relied upon for much more than this simple statement. Wu discusses at length many of the factors that make gene expression analysis unpredictable. Applicant's statement that "differential gene expression which is reproducible, and is correlated with the state of health or disease of the individual does not necessarily result directly in the state of the disease of the individual" is attorney argument which is not supported by evidence on the record. Even if the changes are a result of downstream effects of the pathogenic process, they are related to the state of disease in the individual. Applicant points out that certain prostate

markers were used as biomarkers without an understanding of their function. The examiner is not trying to require an understanding of ABCA1 in CAD or any other disease, nor does Wu suggest that such is necessary. The examiner is looking to the specification for adequate guidance for making and using an invention in a highly unpredictable field of endeavor.

Applicant states that the results of Cheung et al. cannot be reliably extrapolated to primary blood samples since Cheung et al. are using cultured cell lines. However, this is irrelevant to the point of Cheung et al. which is that among individuals (in this case cell lines) there is natural variability in gene expression for any particular gene. Attorney arguments are not sufficient to establish that this biological fact is not the case. Applicant further states that to the extent that Cheung et al. could still be considered to suggest that larger populations of diseased and control populations may be useful, this experimentation is routine. However, as previously noted, for the reasons discussed, this is not routine experimentation given the lack of guidance in the specification, the lack of working examples, the high degree of unpredictability in the art area and the other factors discussed. In the absence of the critical disclosure of the specification and the unpredictable nature of the technology, the further experimentation is inventive- applicant has provided one of skill in the art with an invitation to discover the actual relationship between ABCA1 expression in the blood and CAD.

The instant situation differs tremendously from In re Angstadt, wherein a large number (forty) examples were provided, only one of which did not work. In In re Angstadt, the court determined that there was sufficient guidance in an unpredictable art. The court further stated, however, that "each case must be determined by its own facts." The facts of this case do not support an enabled use for the claims, for all of the reasons discussed in the rejection. Here, the

situation is quite different because the specification does not provide data or guidance sufficient to support the claims of any embodiment of the claimed invention, let alone multiple embodiments.

Conclusion

15. No claim is allowed.

16. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Juliet C Switzer whose telephone number is (571) 272-0753. The examiner can normally be reached on Monday, Tuesday, or Thursday, from 9:00 AM until 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached by calling (571) 272-0735.

The fax phone numbers for the organization where this application or proceeding is assigned are (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571)272-0507.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of

the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

/Juliet C. Switzer/
Primary Examiner
Art Unit 1634

March 21, 2008